A REPORT FROM THE AMERICAN ACADEMY OF MICROBIOLOGY

THE FUNGAL KINGDOM

diverse and essential roles in earth's ecosystem

AMERICAN SOCIETY FOR MICROBIOLOGY

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EXECUTIVE SUMMARY

There's more to fungi than just mushrooms. Fungi are the cause of scores of life-threatening diseases, they are the earth's best degraders of organic matter, and they are proving to be more useful to science and manufacturing every year. They come in many forms, ranging from single-celled yeasts on the order of ~10 ÌM to mushrooms the size of dinner plates to thin, powdery coatings of mold. Despite the diversity that science has revealed about fungi and their myriad roles in health, ecology, and industry, much about these organisms remains a mystery.

The American Academy of Microbiology convened a colloquium November 2–4, 2007, in Tucson, Arizona, to discuss fungi, the current state of research in fungal biology (mycology), and the gaps in our understanding of this important group of organisms. Experts in mycology, medicine, plant pathogens, genetics/ genomics, ecology, and other areas developed specific recommendations for advancing fungal research.

Recent studies have revealed that fungi are more closely related to animals than many other eukaryotic organisms, and these two successful kingdoms diverged from their last common ancestor (a unicellular organism that lived in the oceans propelled by a flagellum) on the order of a billion years ago. Thus, fungi have a great deal to teach us about more complex organisms, including ourselves. The fungal kingdom comprises at least eleven separate groups (7 phyla plus 4 subphyla of the polyphyletic Zygomycota) with diverse genetics, morphologies, and life histories. Genomics (research that uses all or part of an organism's genetic material to learn more about that organism) has opened many new windows on the fungal world, but future progress in fungal genomics will likely be hindered by challenges in fully exploiting the sequence data. Continued support for fungal genomics is critical considering the real possibility that genome sequencing will eventually lead to improvements in the detection, prevention, and treatment of fungal disease. Colloquium participants agreed that the highest priority is to establish a Fungal Genomes Database to assemble and organize genome sequence data, annotation data, and links to the relevant literature.

Research that records which genes are expressed (transcriptional profiling) is also revolutionizing fungal research, and comparing gene expression of the same fungus under different conditions, or different fungi under the same conditions, will be essential to preventing and treating fungal disease. Now that transcriptional profiling is moving from array-based technology to sequence technology, these data have become portable in the sense that results from one laboratory can easily be compared to those from another by computation. To facilitate these comparisons, a central database is essential. Finally, environmental sequencing (metagenomics) has begun to impact fungal biology, and these data will need to be accommodated in a database to make it possible to compare fungal communities across different environments.

A global fungal census, which would describe the various locations and species of fungi that exist in nature, is recommended. Fungi are present in every ecosystem where they perform many tasks, including biomass degradation and participation in mutually

beneficial symbiotic associations with plants. And, like any other organism, fungi may behave very differently in the wake of ecosystem disturbance or when introduced in a particular ecosystem from other regions of the world, even causing outbreaks of disease in humans and plants. In recent history, humans have been responsible for initiating a number of fungal invasions into new geographic regions, sometimes with devastating consequences. More research is needed on fungi in the environment—their diversity, their numbers in different locations, their functions—before scientists can effectively manage introduced fungal diseases or cope with fungal contamination in homes and businesses. A global fungal census, which would describe the species of fungi that exist in nature in the context of their habitats, is recommended. It also remains unclear whether there are fungi whose presence promotes the health of the host, as has been shown for some members of the bacterial component of an animal's microbiota.

Not only is the fungal global ecology of interest, but also the human fungal ecology. It is a little known fact that many different fungi live on and in the human body. Of the estimated 1.5 million fungal species that exist today, more than 200 species have been associated with humans, either as pathogens or as commensal organisms that apparently do us no harm. A census of the fungi associated with the human body is necessary to promote understanding and lead to ways to prevent human disease and is highly recommended. Although many fungal diseases are associated with patients with depressed immune systems, some fungal infections, including sinusitis and vaginitis and athlete's foot, strike healthy people, and fungi may also be linked to asthma, allergies, and sick building syndrome. Given that the incidence of fungal diseases is on the rise, it is also important to continue developing new vaccines and new types of treatments for overcoming these diseases. Diagnostics for fungal diseases are utterly inadequate when, in fact, accurate and early diagnosis of fungal diseases is critical for managing disease and saving lives. New and more rapid techniques are needed for diagnosing fungal infections.

Fungi synthesize a seemingly limitless array of secondary metabolites, including many antibiotics, such as cyclosporin A, a revolutionary drug that inhibits the rejection of transplanted organs, and statins, which are widely used to treat elevated lipid and cholesterol levels and reduce the risk of heart disease. Many fungi and fungal enzymes are used in industry in processes ranging from fermentation to food production to the conversion of biomass to ethanol. Further genomic sequencing of key fungi promises to provide additional insights into the nature and synthesis of novel biologically active natural products and untapped metabolic potential.

Certain fungi can infect both plants and animals, overcoming significant differences between these very different hosts. Known trans-kingdom pathogens include Aspergillus flavus, which infects and damages oilseed crops, insects, and humans. Pathogenic fungi are dangerous potential biological weapons because they are inexpensive to acquire, and many are easy to culture in large quantities. The potential for fungi and fungal toxins to be used as weapons is seriously underestimated and understudied. The field needs advocacy to raise the consciousness of fungi as potential bioweapons and ensure that this kingdom benefits from bioterrorism-related funding.

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RECOMMENDATIONS

1. Create a Fungal Genomes Database
Researchers involved with fungi must focus efforts on developing a comprehensive fungal genomics database in order to make the vast quantities of sequence data more available and to enable the field to fully capitalize on the promise of genomics. The database should include fungal genome sequences, including sequences acquired by "low pass" sequencing, annotation and curated gene information, and links to the relevant literature.

2. Conduct a Global Fungal Census
With respect to environmental fungi, a global census of the various locations and species of fungi that exist in nature is a research priority. A census of this kind is critical to our ability to establish the role of these fungi in normal and perturbed ecosystem function.

3. Conduct a Census of the Fungi in the Human Microbiome
It is important that researchers conduct a census of the fungi associated with the human body under a variety of conditions. A fungal census of the human microbiome would pay abundant dividends in understanding and preventing human disease.

4. Develop New Drugs, Diagnostics, and Therapies
Researchers must explore new methods for addressing fungal infections, including new antifungal drugs, improved diagnostics, and alternative therapeutic methods like immunotherapy and antibody therapy.

5. Investigate the Roles of Fungi in the Pathogenesis of Asthma and Allergies
Studies must be designed to address unanswered questions about how fungi relate to asthma and allergies. Understanding both the normal fungal biota of the human body and the normal biota of our surrounding environments may shed light on whether fungi (or possibly combinations of fungi) cause these problems.

6. Increase Training in Fungal Physiology and Classical Mycology
There is a clear need to train more fungal physiologists and classical mycologists (systematists) in order to maintain a pool of expertise in these fields. Mycology must retain its knowledge base in classical mycology, lest scientists be forced to rely solely on molecular typing to identify environmental and clinical fungi, a technique that can give results that are inconsistent with biology.

7. Sequence More Fungal Genomes
Researchers should prioritize fungal genomes for sequencing. It is important to have representative sequences from each of the fungal phyla, but it would also be very useful to have selected groups of fungi that are particularly well sampled so that scientists may begin to understand the forces that drive evolution.

8. Evaluate the Impacts of Mold in Homes and Businesses

More effort should be devoted to testing and long-term monitoring of mold contamination and human health in New Orleans and other areas flooded during hurricane Katrina. Natural disasters like hurricane Katrina provide natural laboratories for understanding the effects of perturbation on fungi and the subsequent impacts of fungi on human health.

9. Report and Track Fungal Infections

Public health agencies should implement formal programs to report cases, track disease progress, and design interventions in outbreaks of fungal disease. The lack of reporting and tracking systems has made it difficult to control the spread of fungal pathogens because good epidemiological data on the scope of infection is usually not available.

10. Carefully Consider the Role of Fungi as Potential Biological Weapons and Emerging Infectious Diseases

Colloquium participants felt that the fungal kingdom was being largely ignored for its potential to provide devastating new pathogens, either by natural process or by nefarious activities, such as biological warfare agent development. A reevaluation of the potential role of fungi as biological weapons and emerging infectious diseases could translate into additional interest and resources.

INTRODUCTION

What would the world be like without fungi? What if fungi didn't degrade dead plant material or help to manufacture many of our most indispensable antibiotics, medicines, and vaccines? What if fungi did not live on and inside our bodies? And what if fungal diseases didn't stalk crops, wildlife, and humans alike?

Mycologists—scientists who study fungi—find it difficult to envision a world without fungi, because they know these organisms are integral to almost every facet of ecology, agriculture, and medicine. However, most people, including many scientists, are largely unaware of fungi and the roles fungi play in the world around us.

What are fungi? Fungi are eukaryotic, heterotrophic organisms (they consume organic forms of carbon for energy). They come in three basic shapes: unicellular yeasts, filamentous hyphae (molds) and, among the most basal groups, flagellated, swimming, unicellular organisms that encyst to form sporangia. The yeasts, hyphae, and sporangia have cell walls that contain at some of the rigid polysaccharide chitin, along with a variety of glucans. If you think you know what a fungus looks like, you may want to think again. Fungi are wildly diverse in appearance, ranging from the well-known mushrooms and molds to the less familiar smuts, rusts, truffles, yeasts, and others.

In the environment, fungi are the primary degraders of organic matter, responsible for turning dead plants into the small nutrient building blocks other organisms can use. In tropical rain forests, ~50% of the dead plant and animal matter (by weight) is degraded by fungi. Without fungi, dead plants wouldn't break down promptly, and dead material would gradually accumulate, eventually choking off living plants. Mycorrhizae represent another way for fungi to impact ecosystems. These associations, in which fungi live intimately with plant roots, allow plants better access to nutrients in the soil and provide these plants a competitive advantage over plants that lack mycorrhizal fungi. Almost all vascular plants interact with mycorrhizal fungi, and some, such as orchid species, are totally dependant on their fungal partner to germinate and grow. Reforestation requires special mycorrhizae to be successful. In the rumens of cattle and other livestock, fungi help bacteria symbiotically by breaking down cellulose that bacteria can then degrade into even smaller molecules so that the animal can harvest energy from cellulose-rich grasses.

Certain fungi are also responsible for causing disease in humans, plants, animals, and insects. In humans, fungal diseases, which are also called mycoses, can range from merely aggravating (athlete's foot) to life-threatening (*Candida albicans*, *Aspergillus*, and *Cryptococcus*), and perhaps because the incidence of fungal disease is underreported, they are more much common than most people think. For example, each year about 200,000 Americans contract coccidioidomycosis—20,000 seek medical treatment, 2,000 are hospitalized and 200 die. There are approximately 5,000 cases of cryptococcosis per year, and in Africa and Asia, as many as 30% of AIDS patients are afflicted with cryptococcosis. Fungal molds

have also been implicated in sick building syndrome, in which building occupants experience a range of illnesses or discomforts upon exposure to a given dwelling. And fungi are suspected to potentially play a role in asthma as well.

Most plant diseases are caused by fungi making these organisms very important in agriculture. The repercussions of managing fungal pathogens on crops—the money and effort spent, the numerous pesticide applications, the consequences of these applications for surface water and soil quality, and the impacts on crop yields—are extraordinary.

Notwithstanding their negative impacts on health, fungi are invaluable resources. Their value is particularly clear when you consider the usefulness of the many secondary metabolites fungi produce. Secondary metabolites are compounds that are not necessary for growth and reproduction, and fungi make them for a variety of reasons, including self-protection from predators, such as mites and amoeba, killing competing fungi and bacteria, and signaling to nearby microbes. We use fungal secondary metabolites as life-saving medications (including penicillin, the first antibiotic ever discovered), as well as drugs that facilitate organ transplants (cyclosporine) and that reduce cholesterol (statins), and insecticides. As more fungal species are discovered and characterized, the opportunities to capitalize on new compounds to improve human health or contribute to industrial or biotechnological uses will increase exponentially.

The food industry also relies on fungi to do some of the heavy lifting in manufacturing. All leavened bread, all alcoholic beverages, vinegars, citric-acid-based beverages, the Roquefort and camembert cheese families, and many Asian foods, such as tempeh, soy sauce and miso, are among the many foods produced with the assistance of fungi. Fungi, including mushrooms and truffles, are also used as food sources themselves.

Although fungi have been used traditionally in food and alcoholic fermentations, in more recent times recombinant yeasts have been used to produce biologically-active proteins and other compounds in an efficient and cost-effective way. Due to the fact that yeasts are eukaryotic organisms, the production of proteins with the correct post-translational modifications making them biologically active has proven to be of tremendous benefit in the production of therapeutic proteins. The protein secretion pathway for the yeast *Pichia pastoris* has been successfully humanized through genetics and engineering and enables precise control over modifications of proteins. These organisms also make it easier to produce drugs, biologically active products, diagnostics, and vaccines. Yeasts and other fungi are presently being used as production machines for vaccines, vitamins, monoclonal antibodies for use in immune therapy, and other therapeutics. In the future, industry will undoubtedly expand on current strategies to produce ever more complex molecules with great efficiency and accuracy, reducing the costs of many therapies currently in use today.

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FUNGI IN THE ENVIRONMENT: VITAL ROLES AND THREATS

Despite the ubiquity of fungi and their pervasiveness in biology, very little is known about the extent to which fungi reside in various niches. From the degradation of organic matter to mycorrhizal associations, ecosystem health is inextricably linked to the activities of fungi, and these roles can be interrupted in unforeseen ways when the environment is disturbed.

New energy and focus is needed for describing and cataloguing fungal diversity in the environment. We recommend conducting a global fungal census to uncover more information about the distribution and variety of fungi in their various niches. The census should also address the effects of ecosystem perturbation on fungal behavior.

NON-PATHOGENIC ACTIVITIES

Fungi are often overlooked in ecology, but their influence in the environment and their roles in biology are undeniable. Ecosystems rely on fungi to degrade organic matter and mineralize the products, making essential elements available for uptake by plants and bacteria. In broader terms, these fungal degradation activities have a profound influence on the carbon cycle, particularly with respect to degradation of lignin, cellulose, and other plant materials and the subsequent release of carbon dioxide into the atmosphere. In addition, many crops, forests, and other ecosystems rely on interactions with fungi to provide nitrogen and other nutrients. Roughly 60% of plants interact with fungi in ways that benefit the plant. Fungi are also consumed by many organisms, including animals, insects, worms, bacteria, and other fungi.

Fungi maintain a myriad of different symbiotic relationships with animals, plants, insects, amoeba, bacteria, and other fungi. The human gut, which is teeming with microorganisms, is one fungal habitat that is of particular interest to humans, but even here we know relatively little about the interactions between fungi and the host and between fungi and bacteria. Other key interactions include mycorrhizae, symbiotic associations in which fungi colonize the roots of plants and provide a linkage between plant and soil, an arrangement that often grants a plant a competitive advantage over plants that lack mycorrhizal fungi and grants the fungus much-needed carbon. Plants would not have been able to colonize land without mycorrhizal fungi, and most species of plants today continue to engage in these associations to extract nutrients from their environments. Bacteria, too, maintain symbioses with fungi. Certain fungal plant pathogens, for example, play host to endosymbiotic bacteria that regulate or enable the production of virulence factors and permit the fungus to infect key crop plants like rice or allow the fungus to produce spores during its lifecycle for dissemination and survival.

THE IMPACT OF ECOSYSTEM CHANGE ON FUNGI

Fungi as integral parts of diverse ecosystems are subject to the same kinds of stresses other populations experience during ecosystem change. Events and processes such as invasions, migration, climate change, outbreaks, or land use change (such as forest-to-agriculture transitions) can put ecosystems under stress and induce fungal imbalances as measured by disease prevalence, sampling, or even direct observation.

Example situations in which fungal populations have been manifestly impacted by changes in an ecosystem include:

- **Black mold in the ecosystems around Chernobyl, Ukraine.** In the wake of the 1986 disaster at the Chernobyl Nuclear Power Plant, areas surrounding the former power plant were contaminated by significant levels of radioactivity. These areas have seen increases in the incidence of a form of black mold that is destroying plant life and eroding environmental quality. Scientists hypothesize that melanin protects the mold from the radioactivity, but it may also serve as an energy-harvesting apparatus.

- **Coral bleaching.** Bleaching of corals due to ocean water temperature elevations promotes infection by *Aspergillus* species, resulting in coral death.

- **A rise in *Cryptococcus gattii* infections.** It has been suggested that *C. gattii* takes advantage of some genetic changes and possibly alterations in its ecosystem, and that recent dry conditions during the summers on Vancouver Island have encouraged an outbreak of *C. gattii* infections in the area. Prior to the outbreak of cryptococcal disease in British Columbia, Canada, the environmental niche of *Cryptococcus gattii* was thought to be restricted to tropical or semi-tropical climates. *C. gattii* as the causative agents of an emerging infectious disease is an example of the role of micro-environmental changes, including climate change, that will increase the potential for exposure to pathogenic fungi to humans, domestic and wildlife. How the organism came to Vancouver Island and is now spreading beyond to the British Columbia mainland and into the United States in Washington and Oregon cannot be known for certain, but likely has involved human activities.

- **Mold in homes.** Wet indoor environments encourage the overgrowth of *Stachybotrys* species and other molds, which has been associated with sick building syndrome. One prominent example of mold overgrowth can be found in homes and businesses flooded during hurricane Katrina in August 2005. Very little is known about the impacts of indoor mold growth on human health, but it is possible the effects of mold exposure are profound and lasting. The health impacts of mold contamination in flooded portions of New Orleans and surrounding areas cannot be predicted. Should affected houses be destroyed or rebuilt? Is any exposure to indoor mold unacceptable? The answers to

these questions are not known. Natural disasters like hurricane Katrina provide natural laboratories for understanding the effects of perturbation on fungi and the subsequent impacts of fungi on human health. More effort should be devoted to testing and long-term monitoring of mold contamination and human health in Katrina-affected areas.

- **Mold in the space station.** Air in space vehicles is often contaminated with fungal spores. Mold has become a major problem for the Russian space station Mir, where it has been found growing in sensitive electronic equipment.

- **Fungal outbreaks following earthquakes.** Outbreaks of *Coccidioides* infections have occurred in California following earthquakes in the 1990s that disturbed soil leading to airborne spores and infections in humans.

It is common knowledge that global climate changes are constantly occurring and are probably more pronounced in our current era. These changes will impact the ecology of fungi and their relationship to the environment before we have been able to identify them and assess their role in current ecosystems. Global climate change will result in profound ecological alterations, and its impacts on fungi will inevitably affect humans as well. One example of these impacts that has already been witnessed is the emergence of fungal resistance in certain plant cultivars that were once sensitive. Also, in some areas of the world, fungal plant pathogens are spreading into new territories, and monoculture approaches to agriculture may prove to be particularly susceptible to outbreaks. If scientists can learn how ecosystem changes predispose an environment to fungal threats, they can prepare proactively for these threats in the future, rather than react to crises as they arise.

As illustrated above, environmental changes can and do bring about changes in fungal activities, but it is also possible these changes could be so profound as to threaten the existence of a fungal species. However, there is little information on the robustness of fungal populations in the environment, so the possibility of extinction is difficult to gauge. In Europe, some mushrooms are considered endangered, and in Eastern Europe acid rain has caused declines in mycorrhizae, but mushrooms and trees may be impacted by any number of stresses, and cause and effect are difficult to tease apart in these situations. Is a decline in mycorrhizae causing a decline in forests or vice versa? This has yet to be established. It may be possible to use fungi as biomarkers for environmental stress or as indicators of ecosystem health, but currently the links between various species of fungi and ecosystem health are vague.

FUNGAL DISPERSAL AND INVASIONS

The introduction of fungal species into the environment is often traced through the spread of fungal disease. Fungal spores can travel widely by air currents, and transcontinental spread of spores is known to occur. Although fungi can disseminate by means of wind, insects, and seeds, in recent history human activities have

been primarily responsible for introducing a number of fungal diseases into new geographical regions, and these introductions have sometimes caused serious problems. Examples of human-mediated introductions include:

- **The "chytrid invasion."** Humans have transported the African clawed frog, *Xenopus laevis*, all over the world for use in pregnancy tests and research. This frog species carries with it a species of fungus, *Batrachochytrium dendrobatidis (Bd)*, a chytridiomycete, to which *X. laevis* is resistant but many other amphibians are not. The fungus is responsible for severe declines in the populations of amphibian species around the globe. Studies of population genetics of the pathogen, so far, do not confirm (or refute) the spread of chytridiomycosis by *X. laevis*. The American bullfrog (*Lithobates catesbeiana*) is another *Bd*-resistant candidate for spreading this disease. Although it has been shown that *X. laevis* carries the disease now, it is not known if it was the first to do so.

- **Crop disease.** All, or almost all, crop diseases are transported throughout the globe by human activity.

- **Tree diseases.** Dutch elm disease and chestnut blight were both spread by human activities from areas where trees had evolved some level of resistance to areas where most trees were sensitive to the respective fungal pathogens. In recent times, pitch canker, which is destroying the native stands of Monterey pines, and sudden oak death, which is decimating coastal oaks on the Pacific coast and caused by the pseudofungus (Straminopile) *Phytophthora ramorum* and spread by nursery trade, are among many invasive fungal species transported by human activity.

- ***Cryptococcus gattii* and *Eucalyptus* trees.** *C. gattii*, which causes cryptococcosis in humans, is primarily associated with Australian *Eucalyptus* trees and has likely been spread along with these trees or materials derived from them when used in plantation and reforestation programs in Africa, Southeast Asia, South and North America, India and parts of Europe. As one example, in the 1800s thousands of eucalypts were imported from Australia, where *C. gattii* is endemic, and planted to stabilize the soil in parks and land throughout California, and in some cases the organism has been recovered from trees in these new locales.

- **Rose handler's disease.** *Sporothrix schenckii*, which causes sporotrichosis (also called rose handler's disease), was widely dispersed when infected plant seedlings and their sphagnum moss packing were shipped from one area to another.

- **Human travel and fungal diseases.** Human movement can carry human fungal diseases from areas where a disease is endemic to new territories. *C. gattii*, for example, has spread from Vancouver Island, where an outbreak began in the late 1990s, to Washington State, thanks in part to the movement of vehicles and travelers.

scientists hypothesize that melanin protects the mold from the radioactivity, but it may also serve as an energy-harvesting apparatus.

- Molecular analysis suggests that humans may have been inadvertently responsible for the introduction of *C. immitis* into the South American continent as they migrated into that region in Paleolithic times. Furthermore, coccidioides species may infect travelers who visit endemic areas even for short periods of time.

- Nosocomial spread of *Candida* species on the hands or nails of health care workers to patients in intensive care units.

- Transmission of endemic mycoses with organ transplantation, leading to cases of infection in patients with no travel history to endemic areas.

In cases in which pathogen dispersal is aided by human travel, interventions could conceivably be designed to contain the spread, although this would require increased knowledge of ecology and pathogenesis or avoidance of aerosols of dust in southwestern U.S. deserts. Another example is *Synchytrium endobioticum*, a chytrid disease called black wart of potato, which is on the U.S. bioterrorism list. Strict controls are in place to keep this pathogen from spreading out of Newfoundland. When this fungus has escaped, it caused the U.S.-Canada border to be temporarily closed to potatoes. http://maine.gov/agriculture/pi/whatsnew/potatowart_CA.htm

Historically, fungal diseases have not been reported or tracked. This has made it difficult to control the spread of fungal pathogens, because good epidemiological data on the scope of infection are not consistently available. Fungal infections are reportable in France since 2002; the French National Reference Center for mycoses and antifungals is charged with surveillance. Other public health agencies should implement formal programs to report cases, track disease progress, and design interventions in outbreaks of fungal disease. For effective management of fungal invasions, there needs to be a system for data collection, determination of the scope of the outbreak, and implementation of policy. In cases in which pathogen dispersal is aided by human travel, interventions could be arranged around highways and logging routes to try to contain the spread.

A standard protocol in the advent of a specific outbreak of a fungal disease of humans, animals or plants should be in place that will summon the available experts on the subject and make their expertise available to government agencies. In some instances, the necessary information and expertise are available, but for multiple reasons these resources are not utilized for shaping public health policy. A standard procedure should be developed to address outbreaks; interdisciplinary groups of public health officials, zoologists, and microbiologists must address this need.

USING FUNGI AND FUNGAL PRODUCTS IN INDUSTRY

The use of fungi in industry has two aspects to it. The first concerns the application of the enzymatic capacity of fungi to mediate industrial processes. Some of the processes fungi carry out in the environment have been appropriated by industry, and there are many more opportunities for exploiting other fungal capabilities that

remain to be explored. Fungi decompose biomass, and they are particularly effective at breaking down celluloses and five-carbon sugars. Industry can capitalize on these metabolic capabilities by using fungi to produce fuel (including ethanol) from plant materials. In a recent development in this arena, the Biogreen compact in Europe discovered a zygomycete capable of producing ethanol more efficiently than *Saccharomyces*, and waste products from the process can be made into diapers and other products. These fungal degradative capabilities can also be used in bioremediation, the treatment of chemical contamination using microorganisms or microbial enzymes.

The other use of fungi in industry is related to the field of biotechnology. Biotechnology has emerged hand-in-hand with the availability of *Saccharomyces* as a "small factory" for the production of innumerable products that range from hormones to proteins for therapeutic uses. Fungal systems have an advantage over bacterial systems in that they can produce certain products with the correct post-translational modifications needed for activity.

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RESEARCH NEEDS

Environmental sampling from a variety of niches (insects, animals, water, air, soil, and compost piles, to name a few) has revealed a remarkable diversity of fungal species and countless fungi that haven't been named yet. The current level of scientific knowledge about fungi is inadequate for managing exotic and introduced fungal diseases in the environment, for preventing the possible endangerment or extinction of species of fungi, or for coping with fungal contamination in homes and businesses. This shortfall in knowledge extends to even the most fundamental issue: we do not know where the known species of fungi live or how many are present in those niches, so it's difficult to appreciate how fungi interact with and influence their environments. With respect to environmental fungi, a global census of the various locations and species of fungi that exist in nature is a pressing research priority. A census of this kind is critical to our ability to establish the role of these fungi in normal and perturbed ecosystem function.

Global Fungal Census

A global fungal census should entail extensive sampling of the environment, followed by PCR amplification using specific primers for fungi; the ITS region of the rDNA genes should then be sequenced. ITS sequences will provide a kind of molecular bar code for identifying fungi within the environmental samples and their relationships to known species.

Diverse ecosystems across the planet should be sampled for this census, including both terrestrial and aquatic habitats. Scientists have recently discovered many novel and diverse species of fungi living in the oceans, for example, proving these habitats and the deep ocean, in particular, are ripe for further study. Fungi associated with insects are also extremely diverse and merit further research. Nearly every insect species examined to date is host to its own unique and diverse microbial community,

FUNGI AND INDOOR AIR QUALITY: WHAT'S THE EVIDENCE?

Sick building syndrome is an important environmental problem, one that may be due to airborne mold spores or toxins. A huge industry has sprung up around diagnosing and treating the problem of "toxic mold," and litigation in these cases is not uncommon. However, there is a serious lack of scientific data to support any stance with respect to indoor mold toxicity or remediation, and, although efforts to understand the relationship between fungal growth and sick building syndrome have begun, a great deal of work lies ahead to scientifically validate its existence. Current studies are limited to cultivatable fungi that sporulate profusely, but there is evidence that uncultivatable fungi, basidiomycetes, for example, contribute to asthma and may be key players in health aspects of indoor air. Here, genetic and genomic studies will be the principal means of identifying and studying the fungi. Sick building syndrome may be a particular problem in New Orleans and other areas on the Gulf Coast that were flooded by hurricane Katrina in 2005. Indoor mold growth is rampant in these regions, and individuals and institutions alike have associated fungal growth with respiratory disease in the absence of conclusive medical evidence. This deficit should be addressed promptly.

The relationship between fungi and allergic respiratory conditions, including asthma, is also poorly understood. The asthma rate among children in the U.S. and elsewhere is skyrocketing, and doctors and parents alike are struggling to find the causes of the epidemic. There is evidence that some cryptococcal lung infections may alter the immune polarity of the lung to predispose towards the development of pathologic lung responses such as those seen in asthma. Recent work suggests fungi may be to blame for many cases of allergic respiratory distress, but current evidence and research data are anecdotal and are not sufficient for prescribing treatment plans for patients or remediation programs for their workplaces or homes. There is a great need for scientific scrutiny in this field.

and these communities are likely to include many new fungi. The fungi associated with mites could point the way to new biocontrol measures for mite pests, and beetles are likely to be a significant source of diverse yeasts, to name a couple of the potential avenues to explore. Tritrophic interactions of insects, fungi, and plants could be a good place to seek novel fungal antimicrobials. Animal gastrointestinal tracts contain numerous species of fungi, but these niches have been largely unexplored. Extreme environments, including hot springs, thermal vents in the oceans, and arctic ice, are also a mystery with respect to their fungal inhabitants. All of these habitats should be surveyed more thoroughly. Research should also explore possible changes in the distribution of fungal populations in response to various perturbations.

Community-level Genomics of Fungal Populations

Although almost all metagenomic, microbial research has focused on bacteria, fungi are beginning to be recognized as major players in natural environments. Researchers involved in these projects suggest that the complexity of the bacterial portion of these communities is so great that analyzing the fungal component as well would make these projects prohibitively complicated and expensive. Of

course, ignoring the fungi means that the studies are incomplete and doubtless miss key environmental players or even the key environmental player. To make environmental, metagenomic studies more valuable, researchers who focus on fungi must become involved in genomics research on microbial communities.

The goals outlined above, namely, conducting a census of the distribution and diversity of fungi in their many habitats and extending community-level genomics work to include fungi, will propel scientific progress in a number of different areas, including:

- Learning more about the fungi that populate the indoor environment will have an important impact on how contaminated environments are managed. Improved, science-driven management will have major social consequences and implications for human health and building codes.

- More knowledge about the distribution of fungi would reveal the niches favored by pathogens, which could help guide doctors in making recommendations for their immune-compromised patients and the environmental exposure risk factors.

- Newly discovered fungi will undoubtedly furnish new clusters of genes for making useful secondary metabolites for use in many industries, since there is much variation among fungal species and even among different strains of the same species. Proteins involved in cellulose and lignin degradation would be extremely valuable in biofuel production.

Researchers need a number of new tools for approaching questions in mycology, including genetic systems, new technologies and approaches for cultivation, model organisms, and good stock collections, to name a few.

FUNGI AND PATHOGENESIS

Fungi may be pathogens of plants, animals, insects, and even of other fungi, but in the context of fungal diversity, there are relatively few pathogens. In humans and animals, a potential restriction on their diversity and numbers is the requirement to grow at the body temperature of the host. Over time, there will be an increase in the number of fungal pathogens of humans due to the aggressiveness, the consequences of modern medical care, in the way that we treat patients in hospitals, and in view of our recent experience with the emergence of more fungi that cause disease. Fungi that infect humans have evolved several times independently, and it will happen again.

In plants, pathogens may grow at a variety of temperatures, depending on the climate favored by the plant and the season in which the fungus grows, so the diversity bottleneck on plant pathogens is less severe. Ninety percent of plant pathogens are fungi.

In molecular terms, it does not take much to tip the balance and turn a harmless fungus into a plant pathogen. Disease is a continuum in plants; knocking out a single gene can turn an endophyte into a pathogen, making the study of these organisms of primary importance because of their impact on economic development. Even mycorrhizal fungi can be pathogens on other plant species—for example, *Rhozoctonia solani* is mycorrhizal with the Australian Green hooded orchid, but it can also parasitise crop plants—and thus, when out of the context of the normal host, fungi can cause significant problems.

FUNGI AND HUMAN HEALTH

The human "microbiome" includes all the microorganisms present on or in the human body; fungi are a minority constituent of this microbial community, numerically speaking. It's difficult to say how many fungi are associated with the human microbiome, but the number and species of fungi on and in a given human are likely to change with climate, diet, gender, age, immune status, prescription medications, and other factors. Recent surveys of bacteria associated with the human body show that these populations shift over time and are highly variable from person to person and, therefore, support this hypothesis.

Of the roughly 1.5 million fungal species estimated to be alive today, only some 200 have been associated with the human body so far, either as pathogens or as commensals, and of these, a dozen or so represent the most common fungal pathogens. This includes the *Candida* species *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. metapsilosis*, *C. orthopsilosis*, *C. krusei*, *C. guilliermondii*, *C. lusitaniae*, *C. keyfr*, *C. dubliniensis*, and *C. glabrata*, which are components of the normal microbiota and can also cause superficial and systemic infections; molds (such as *Aspergillus fumigatus* and zygomycetes) that infect the lung; the basidiomycete

fungi *Cryptococcus neoformans* and *C. gattii*, which infect the lung and central nervous system; and the dimorphic human fungal pathogens, of which there are several species (*Histoplasma capsulatum*, *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, *Coccidioides immitis*, *Coccidioides posadasii*, *Penicillium marneffei*, and *Sporothrix schenckii*). Other fungi commonly associated with human disease include the *Malassezia* species, common commensals that have evolved to specifically interact with human skin and secretions. In turn, these fungi secrete antigenic proteins that may play a direct causative role in skin disorders. Another successful group of human fungal pathogens are the dermatophytes, which cause athlete's foot and other common skin, nail, and hair infections. Finally, there are the black yeasts, *Mucorales*, *Fusarium*, *Scedosporium* and the black molds.

A great deal about human fungal pathogens remains unknown. Researchers do not know, for instance, how many fungal clones (genotypes/isolates) are present in an infected patient, and many of the details about the interactions between fungi and their human host, whether during commensal growth or infection, are not known. Much of the information about pathogens comes from experiments that tested cultures of fungi during logarithmic phase, a growth habit that is poorly reflective of growth in a host. Certain secondary metabolites, for example, function during infection, but secondary metabolites are generally not produced during log phase growth, so studies that use fast-growing cultures will often miss the effects of secondary metabolites.

Fungi do not restrict themselves to causing diseases in immune-compromised or otherwise debilitated hosts, although this is a common misconception. There are many known fungal infections that strike apparently healthy people, including fungal sinusitis and vaginitis, and there may be connections between fungi and such common conditions as asthma, allergies, and sick building syndrome. Even disseminated cryptococcosis can occur in hosts without an underlying disease or disorder. It is also important to note that infecting a human is not necessarily an evolutionary dead end for a fungus. Anthropophilic dermatophytes, for example, are transmitted from human to human, and as such have evolved to cause chronic diseases with little host immune response that are difficult to treat and completely resolve. Commensal fungi like *Candida* and *Pneumocystis spp*. are also passed from person to person, and they have co-evolved with humans and do little damage in their commensal roles. One factor involved in fungal interactions with humans is temperature—commensals must evolve to be able to thrive and grow at 37°C. There are probably other selective pressures, such as access to nutrients. *Malassezia* sp. are associated with human skin as commensals and play a role in common skin disorders, such as dandruff and eczema. Recent genomic studies reveal they lack the enzyme fatty acid synthase, explaining their requirement for lipids during laboratory culture and their known predilection to survive on human skin by scavenging lipids from sebaceous secretions.

It is important to note that, unlike viruses and some bacteria, person-to-person transmission of invasive fungal disease is rare. Since fungi are a minority of the microbes in the human body, it likely that their most important interactions are with their bacterial competitors. Examples of these exchanges are emerging from studies of the interplay between fungi and pathogenic bacteria (*Pseudomonas* species), probiotic bacteria (lactobacilli), and other cohabitants (streptococci).

TRANSKINGDOM PATHOGENIC FUNGI

Fungi, as stated previously, are among the most important degraders of organic materials in the environment, yet as such they sometimes do not have a specific preference for the source of organic matter. Certain pathogenic fungi infect members of both the plant and animal kingdoms, overcoming significant differences between these types of hosts. Fungi that do not require a living host are more likely to pose a threat across kingdom lines, since their requirements for growth are not as specific as those of a fungus that needs the support of living host cells. Many fungi will consume resources regardless of whether the source is alive or dead; they are not selective. Factors that may determine the possibility of fungi to constitute a transkingdom threat include their ability to grow at higher body temperature and the ability to breach surface barriers on insects and plants, and sometimes knocking out a single fungal gene can turn a harmless endophyte into a pathogen.

Fungi that can infect hosts from different kingdoms may develop the ability to infect a given host by training and developing traits using another, related host. Amoeba, for example, are a kind of "boot camp" for *Cryptococcus* species, since amoeba internalize *Cryptococcus* in much the same way human macrophages (attack cells of the innate immune system) do.

High exposure to certain fungi may contribute to their ability to cross kingdom boundaries. There have been reports of farm workers becoming infected with the plant pathogen *Fusarium* after repeated exposure to that fungus.

Almost all fungi, including those that pose transkingdom threats, live in the environment at some point during their life cycle, and their time in this ecological niche could teach researchers about a fungus' vulnerabilities. Known transkingdom pathogens include *Aspergillus flavus*, which infects and damages oilseed crops, insects and humans; *Fusarium* species, which infect cereal crops and immune-compromised humans (and pose a particular public health threat due to their resistance to many antifungals); *Sporothrix* species, which infect both plants and animals; black fungi, which can infect cacti and humans; and Microsporidia, Zygomycetes, *Alternaria* species, and *Cladosporium* species. *Cryptococcus* species may be able to cross kingdom boundaries, but it is difficult for them to infect plants. Other transkingdom fungal pathogens may be waiting to be discovered. Fungi probably have many interactions with different kingdoms of organisms, including fish, frogs, insects, amoeba, mammals, and plants.

MODEL HOSTS

It is important to focus studies on current, well established heterologous host model systems, but it may also prove useful to develop new model systems for studying fungal pathogenesis. Researchers have used models such as *Caenorhabditis elegans* (a nematode), fruit flies, *Arabidopsis* species (plants), frogs, toads, and others, but selecting the right model system is important, and more model systems should be developed. An ideal model is manipulable, tractable, available, reproducible, and inexpensive to purchase and study. Many model hosts have innate immunity, but fewer hosts, including zebrafish and mammals, have adaptive immunity.

VACCINES AND THERAPEUTICS FOR FUNGAL INFECTIONS

Developing new vaccines and therapies for fungal diseases is a neglected area of research. Given the increasing incidence of fungal infections, it is important to develop new vaccines and new types of treatments. The antifungal therapies available today should also be improved.

In some parts of the world, endemic mycoses remain a problem for otherwise healthy, immune competent hosts. South American blastomycosis (caused by *Paracoccidioides brasiliensis*), is a target ripe for vaccine development. There have been significant strides in understanding immunology for other endemic fungal infections, including histoplasmosis and blastomycosis; this progress offers lessons for moving forward with vaccines in the future.

Some fungal diseases are better suited than others to control with vaccines, like coccidioidomycosis, which is limited to distinct geographical regions (the desert southwest of the United States and portions of South America) and poses a particular threat to known subpopulations (including the elderly, new residents, and prison inmates) and would be relatively easy to implement in the community. Likewise, implementing vaccination programs for fungal diseases that strike immune-compromised patients (including *Candida*, *Aspergillus*, and *Cryptococcus neoformans* infections) would also be straightforward, since immune-compromised individuals are usually under the care of a physician and, as such, are easily identified as targets for a vaccine prior to beginning an immunosuppressed state of treatment.

In other cases, it may be difficult to identify members of the target population for a vaccine, making it harder to implement a vaccination program. The effectiveness of adjuvants in vaccines for fungal diseases requires a great deal of study. Also, clinicians need more information about the appropriate length of time to treat fungal infections with antifungal drugs and the better use of fungal biomarkers in management strategies.

Researchers must explore new methods for addressing fungal infections, including new antifungal drugs, improved diagnostics, and alternative therapeutic methods like

immunotherapy and antibody therapy for delivering radiation to the target pathogen. It may also be possible to develop vaccines that deliver cytokine therapy, as well as an immunogen. The success rate in managing invasive fungal infections remains mediocre and does not approach success rates with bacterial infections.

RESEARCH NEEDS

There are many unmet needs in terms of understanding the biology of pathogenic fungi. Filling these gaps in our understanding will have important practical implications for agriculture and medicine, as well as implications for studying the evolution of life. In addition, learning more about fungi and about ways to control and harness them will prove valuable for improving industrial applications like mining fungal genomes and metabolomes for useful chemicals and engineering fungi to produce materials (e.g. vaccines, therapeutic proteins).

Mycology needs access to fast-response funding mechanisms to head off outbreaks. The current outbreak of *Cryptococcus gattii* on Vancouver Island and Puget Sound, for example, demonstrates the need to respond quickly to an outbreak. This required a multi-disciplinary research team to put together the information coming independently from veterinarians, cetacean pathologists, respiratory physicians, epidemiologists, and environmental hygienists to identify an emerging infectious disease and initiate appropriate therapy in order to save lives. Many fungal diseases can muster only small clusters of experts, so assembling a task force to cope with an outbreak of corn blight, for example, would be problematic.

Improved Diagnostics

Accurate and early diagnosis of fungal diseases is critical for managing disease and saving lives, but as many clinicians would agree, diagnostics for fungal diseases are inadequate. Moreover, fungal pathogens are often stealthy and difficult to detect in infected patients at early stages of the disease, when therapies would be most effective.

Techniques commonly employed in the detection of fungal diseases include microscopic examination, culturing, and serology. The utility of these techniques is seriously hampered by lengthy wait times for results and low accuracy. Other approaches, which rely on polymerase chain reaction (PCR), have not been employed to diagnose directly certain fungal diseases (including bloodstream infections involving *Candida* species), and although PCR-based techniques have the advantage of speed, they also suffer significant limitations. Contamination with dead cells released from the gastrointestinal tract, for example, may lead to false positive results in PCR testing, and in biological fluids it is hard to approach sensitivity of cultures. Except for laboratory diagnosis of strains, there are no FDA-approved fungal PCR diagnostic tests yet. Furthermore, the clinical use of identification of fungal infections by metabolomics or proteomics is even more primitive than molecular tests but with great potential.

certain pathogenic fungi infect members of both the plant and animal kingdoms, overcoming significant differences between these types of hosts.

Research laboratories have developed a number of specific molecular tests for fungi, but it is very difficult to integrate these tests into the clinic or field for practical use.

New and more rapid techniques are needed for diagnosing fungal infections. This is an important priority, given that fungal diseases are often treated empirically, without a specific diagnosis. When faced with an unknown fungal infection, doctors are often forced to administer large doses of any of a number of antifungal therapies to their patients without knowledge of the agent responsible, or its drug resistance profile. Blind treatment like this leads to excessive use of antifungal drugs and can encourage the emergence of drug resistant strains. It can also favor the emergence of breakthrough infections with species with decreased susceptibility or resistance to the prescribed antifungal drugs—for example, caspofungin and *C. parapsilosis* and voriconazole and Mucorales. In the developing world, where medical resources are significantly more limited as compared with the developed world, the problem of diagnosing mycoses is even more severe. Certain fungal biomarkers like galactomannan and beta-glucan have been approved for fungal diagnosis in serum, but these tests need better and more consistent integration into clinical monitoring strategies to determine their value.

One prospective idea for new diagnostics that may be fruitful to explore is testing for volatile compounds that are known signatures for particular fungal species using gas chromatography-mass spectrometry. Luminex-based microfluidic-based assays are another possibility for fungal diagnosis (http://www.luminexcorp.com/). It may be possible to develop a single gene chip (like the one available for viral infections) that can be used for diagnosing numerous different fungal infections. A tool like this would allow very rapid diagnosis and could be extended to benefit the ecology, veterinary, and agriculture fields if chips were developed for fungal diseases in wildlife, livestock, and crops.

Methods for diagnosing drug resistance and susceptibility in fungi would also be of value, and predictive and validated detection methods would represent a major advantage in patient care. If there are limited mechanisms of resistance in fungi, resistance could be assayed by analyzing patient samples for the presence of resistance genes. Currently, *Candida* is the only species of fungus for which drug susceptibility information is available. New genome sequence data could provide insights on resistance mechanisms in other important agents, including *A. fumigatus*, *Coccidioides*, and other endemic fungi. Aspects of the molecular basis of drug resistance in fungi remain unexplained. Mapping resistance may require genomic work to uncover the underlying mechanisms and principles.

Investment in fungal research and diagnostics should be scaled appropriately to the magnitude of the problems these organisms pose. For this reason, it is important to collect and curate statistics on the number of patients treated every year

for fungal infections and the economic burden these cases bear for the health care system. Similar statistics are also needed for agricultural pathogens.

Fungal Vaccines

Developing new vaccines for fungal diseases is an important research priority. Many fungal diseases strike immune-compromised hosts, and, hence, are not good candidates for vaccine development, but even immune-compromised patients can sometimes mount a sufficient response to enable them to benefit from a vaccine, and many of these patients can be identified and given vaccine before they enter an immunocompromised state. Immunomodulators could also be used to boost the effectiveness of vaccines in immune-compromised patients.

Molecular biology, genomics, and proteomics can offer new insights into fungal antigens as well as new abilities to produce antigens for use in vaccines. If the role of fungi in eliciting allergic responses were better understood, allergy control could represent a major new application for fungal vaccines.

Funding mechanisms for developing fungal vaccines are weak. In some cases, researchers have made substantial progress developing fungal vaccines, only to be confronted by a lack of funding infrastructure to take these discoveries to clinical trials. It may be necessary to further develop the orphan drug concept to provide a funding mechanism for these projects.

Antifungal Therapies and Other Therapeutic Approaches

As a class of pathogens, fungi are much harder to treat than bacteria because they are eukaryotes closely related to animals [fungi + animals = the opisthokont clade]. Hence imperfectly honed antifungal therapy may harm the fungus as well as the unfortunate patient. Clinicians need new antifungal therapies and techniques for managing disease in their patients. There are a number of new avenues for exploration available. Examples include:

- Immune enhancement, possibly by using an immortalized phagocytic cell line to boost immunity in affected patients,
- Strategies to increase innate immunity,
- Using small molecules that target specific steps of infection, including those that block adherence,
- Using probiotic organisms to enhance the immune response to fungal pathogens,
- Administering strains of fungi with reduced virulence to outcompete virulent strains (although this approach may not work for organisms that also have a commensal role, like *Candida albicans*),

- Delivery of mycoviruses to kill fungal pathogens,
- Better assessment of currently available diagnostic tests and treatments, including combination therapy and the optimal duration of therapy,
- Studies on host susceptibility and responses to infections, and
- A database on clinical and therapeutic issues for all types of mycoses.

Agriculture faces devastating problems with fungal pathogens, but safe and effective agrochemicals and management strategies for preventing and managing these diseases are few. Because fungi are ubiquitous in the environment, management to prevent fungal disease has traditionally involved widespread prophylactic spraying of antifungal chemicals on crops, and this approach has led to groundwater and soil contamination in many places. Researchers need to develop alternative strategies for controlling crop pathogens. Ideas for controlling fungal pathogens on crops include:

- Developing fungus-resistant crops using genetic engineering,
- Using inhibitors of genes involved in formation of melanin, a hard polymer that coats spores of many fungi. Such an approach is used in Asia to control rice blast, a disease caused by the fungus *Magnaporthe grisea*,
- Treating crops with chemicals (e.g., salicylic acid) that induce the plant to turn on natural defense strategies (although this would not be practical on a large scale),
- Enhancing the production of antimicrobial molecules (such as phytoalexins or antibodies produced in plants) in crops to provide defense against pathogens and prevent damage, and
- Using knowledge of fungal genomes to inform farmers about which crop to sow. Identification of fungal avirulence genes enables development of rapid assays for chanes in virulence of fungal populations. Such assays inform pathologists, agronomists, and farmers as to which resistant varieties of a crop plant will be effective at preventing disease each season, and hence which ones should be sown.

CENSUS OF THE FUNGAL PORTION OF THE HUMAN MICROBIOME

Millions upon millions of microorganisms, including many microscopic fungi, are found on and in the human body. However, the role the microbiome plays in human health is poorly understood, and the function of the fungal component is

when faced with an unknown fungal infection, doctors are often forced to administer large doses of any of a number of antifungal therapies to their patients without knowledge of the agent responsible, or its drug resistance profile.

even more elusive. We recommend conducting a census of the fungi associated with the human body under a variety of conditions. A fungal census of the human microbiome has the potential to considerably advance our understanding of how fungi impact human health and disease.

To date, there has been no systematic effort to enumerate and describe the fungi of the human body. There is a clear need for the scientific community to pursue this research. The National Institutes of Health has undertaken a project to study the human microbiome, but these studies are heavily skewed in favor of bacteria, essentially disregarding the fungal component of this important ecosystem.

Before beginning a fungal census, it may be advisable to capture a broad overview of the scope of the matter by analyzing the diversity of the fungal ribosomal RNA genes in the human microbiome to provide a quick view of how many different species of fungi to expect. It is quite possible that many of the fungi associated with the human body will resist cultivation. Once an estimation of the fungal rRNA diversity is available, researchers can begin to design culture conditions to characterize the community over time, space, health, and disease. If two species of fungi are always found together, that would suggest experiments to define the interplay between them.

Sampling sites for the census should include the gastrointestinal tract, the mouth, the skin (including the feet, toes, and scalp), hair, nails, lungs, vagina, and the nasopharynx. The fungal census should include samples from normal, healthy individuals of both sexes from a range of different ages (babies, teens, adults, and elderly individuals), and possibly from individuals belonging to different ethnic groups.

Census studies should also be extended to individuals with diseases, including:

- Patients treated with high doses of antibiotics that are known to perturb the distribution of fungi in patients (these results could shed light on the longstanding hypothesis that antibiotic treatment kills off bacteria and enables fungi to flourish),
- Chemotherapy patients,
- Individuals with skin disorders, including atopic dermatitis, that are associated with different commensal fungi,
- Individuals with depressed immune systems, including AIDS patients and transplant recipients,
- Individuals with metabolic disorder/obesity (an increasingly predominant group in the population), and

- Individuals with exposure to aquatic environments, including sewage, which may increase exposure to chytridiomycetes and other fungi and pathogens.

It may emerge from this census that certain species of fungi are associated with a panoply of diverse, poorly understood diseases, possibly including obesity, autism, alcoholism, irritable bowel syndrome, inflammatory bowel disease, ulcerative colitis, Crohn's disease, malabsorptive syndromes, and colon cancer.

A census of the fungi in the human microbiome would inevitably shed light on the interplay between fungi and bacteria in the gut—interactions that are thought to shape the persistence of bacteria in this environment.

Disease may be more complex than previously thought. Illness may be the product of an imbalanced microbial community. In this scenario, a single pathogen almost never acts alone. Instead, combinations of organisms contribute to illness, and the response of the host bears greatly on the outcome.

A fungal census like the one described here could reveal a great deal about the concept of pathogenicity as it applies to fungi. Fungi can be seen as opportunists waiting for hosts that have the right combination of susceptibilities and ready resources for a fungus to grow and multiply, and some fungi are more aggressive in their pursuit of these host resources than others. A census could enable scientists to name and describe new species of fungi and place them in their appropriate spots on the continuum between "pathogen" and "harmless commensal."

Bacteria are a huge, potentially overwhelming, component of the human microbiota, and they may make it more difficult for researchers to assess the fungal components and contributions in this environment. On the other hand, it is possible that the fungal metabolome will be easier and clearer to discern, despite the minority status of fungi.

Information about the fungal component of the microbiome can be integrated into the emerging systems biology field. Metabolic reconstruction will shed light on which microbes fungi interact with and how.

Study the Links Between Fungi and Human Health

A census of the fungal portion of the human microbiome will uncover a great deal about human health and disease, but many other questions about fungi and health will need to be addressed using more targeted research.

The microbial biota of the human gastrointestinal tract have an important influence on how humans process calories in food. It is natural, therefore, to contemplate the possibility that microbes, and possibly fungi, play a role in obesity. This possibility should be explored.

Fungi may play major roles in asthma and in allergies involving the lungs, sinuses, and skin. For example, there are indications that chitin, and other components of fungal cell walls, may be an important trigger for asthma attacks, and genetic studies have recently linked a human chitinase to asthma risk. Researchers need to design studies to address unanswered questions about how fungi relate to these conditions. It may be that general changes in the microbial flora within the human and in the immediately surrounding environment change the polarization of the immune response. This shift could influence our reactions to fungi in the environment, leading to increasing incidence rates for conditions like asthma. Understanding both the normal fungal biota of the human body and the normal biota of our surrounding environments may shed light on which fungi (or combinations of fungi) cause these problems.

Finally, there is still a great deal to learn about secondary metabolite production in fungi and the possible roles of these products in asthma, allergies, cancer, mental health, and perhaps even longevity.

FUNGAL GENETICS

Scientists have begun to decipher the secrets locked within fungal genes. Genetic sequences are helping scientists improve fungal classification and enhancing our definition of fungal species, leading to ever more clarity and innovation in fungal biology. Genomics, in which scientists study all or part of an organism's genetic material, has enabled considerable progress thus far, but significant work lies ahead, and many more genome sequences are needed. Fungal genetics is an invaluable tool not only for the study of fungal classfication but also for the study of eukaryotic gene evolution and function. A Fungal Genomes Database is strongly recommended for bringing all the current genome data together "under one roof" and improving the accessibility of fungal genomic data to mycologists and scientists of other fields of biology.

The question of which organisms are included within the fungal kingdom has become less controversial. The National Science Foundation has funded the Assembling the Fungal Tree of Life project, in which researchers are conducting a broad survey of the fungal kingdom using a method called multilocus sequence typing analysis and evaluating the relationships among the fungi and closely related organisms based on the similarities in their genetic sequences. This study provides evidence that the fungal kingdom contains at least eight distinct phyla (clusters of closely related organisms), rather than four phyla, as once thought. According to the study (Hibbett et al., 2007), the phyla included in the kingdom Fungi include:

1. Ascomycota
2. Basidiomycota
3. Zygomycota
4. Chytridiomycota
5. Glomeromycota
6. Blastocladiomycota
7. Neocallimastigomycota
8. Microsporidia

The Zygomycota is polyphyletic, meaning the members of this group don't all originate from the same immediate ancestor, so the authors designated the following as subphyla:

- Zoopagomycotina,
- Entomophthoromycotina,
- Mucoromycotina, and
- Kickxellomycotina

FUNGI AND THE SPECIES CONCEPT

As with any other category of organisms, it is important to establish a meaningful and consistent set of criteria for distinguishing one species of fungus from another. Prior to the advent of molecular biology, certain fungi were assigned two names: one for the sexual stage (teleomorph) and another for an asexual reproductive stage (anamorph). This convention is an anachronism that dates from a period in microbiology when, in the absence of molecular evidence, fungi were classified solely on the basis of their morphological form. Dual nomenclature confuses fungal systematics to the detriment of scientific discovery and, most importantly, impairs communication among scientists, hindering the development of the field and the implementation of efficient medical treatment. The difficulties of this system are felt most acutely in education and training and in attracting new talent to the field.

Efforts to assign a single name to each species are now underway, and we strongly encourage further progress. Defining fungal species allows scientists to ascertain fungal mating cycles, breed fungi effectively for desired traits, and ensure accurate representation when sequencing fungal genes. More broadly, a widely accepted definition of fungal species allows scientists to communicate their findings to other researchers and to the medical and agricultural communities.

A species designation should accurately convey the essential characteristics that identify an organism as a member of a particular species. These become particularly important in the case of the clinical environment where the identification of a fungal species is needed for determination of the correct therapeutic approach in sometimes life threatening conditions. With respect to a fungal isolate, clinicians are concerned with the potential properties of the fungus and its drug resistance. Farmers and plant breeders have similar concerns with respect to fungal pathogens in agriculture, but they also need to know about an isolate's dispersal mechanisms and the possibility of host resistance. A detailed analysis beyond a simple species identification is necessary to reveal some of these traits, which can vary between isolates of the same species.

In traditional terms, a species is described as a set of individuals that interbreed, but this is a problematic definition in the case of fungi since sexual cycles are difficult to recreate under controlled conditions necessary to verify species boundaries and the sexual cycles for many fungi have yet to be discovered. As more and more fungi are discovered, it will become increasingly difficult (if not impossible) to determine the ability of these organisms to undergo sexual reproduction in or out of the laboratory setting.

Fungal species, like those of any eukaryote, may be recognized by phenotype (morphological species recognition), genetic isolation (phylogenetic species recognition) or reproductive isolation (biological species recognition. The default method is morphological species recognition, but these taxa typically contain more than one species when phylogenetic or biological methods are applied.

Where mating tests have been possible, good agreement has been found between phylogenetic and biological species recognition. This finding is important because very few fungi can be mated to establish biological species, whereas phylogenetic species can be recognized for any fungus for which DNA can be obtained. However, phylogenetic species recognition is laborious because fungal individuals must be thoroughly sampled throughout their range. As a result, phylogenetic species recognition has been applied only to a relatively few, socially important species—that is, human, animal and plant pathogens, or model organisms. For the vast number of other fungi, we have a morphological species concept and, at best, sequence from the ribosomal DNA region.

Historically, fungi have been identified by observation of macroscopic and microscopic morphology, relying on the production of typical colonial morphology and sporulation patterns.

The most variable of these rDNA regions, the ITS, has been chosen as the fungal "Barcoding" molecule and is useful for assessing fungal identity where well-recognized species identification is not essential (in ecological studies, for example). Following the lead of bacteriologists, mycologists will be able to apply a Bayesian phylogenetic method to add environmental sequences to databases with known sequences and assign the unknown sequences to species with statistical probabilities. Here, ecological studies can be broadened to include socially-important aspects such as quarantine or epidemiology of plant and animal pathogens. Essential to this approach will be a central, curated database of sequence from laboratory and environmental studies. These data will facilitate any method of rapid identification, from the current microarray technology to computational methods that will be applied to high throughput sequencing.

When an isolate's detailed genetic sequence data is not available, it can be difficult (if not impossible) to know whether the fungus is a representative of a known species or something new altogether. Historically, fungi have been identified by observation of macroscopic and microscopic morphology, relying on the production of typical colonial morphology and sporulation patterns. Most of fungal identification in the clinical environment still depends on this approach that requires a specially-trained personnel because not all isolates present the typical structures that enable reliable identification. Nevertheless, DNA-based identification of fungi with the advent of molecular biology techniques presents itself as a more reliable, fast and accurate method for fungal identification. In order to achieve this goal, a fungal typing chip that allows rapid identification would be very useful for researchers, clinicians, and agricultural interests. Ideally, such a chip would allow users to identify a fungal isolate and place the isolate in the context of a detailed fungal database that provides information about the relevant characteristics of each organism. A detailed database would also aid in the classification of nonculturable fungi uncovered by surveys of fungal diversity. To assemble such a chip and an accompanying database, it is necessary to identify key fungal sequence signatures that could be used for isolate identification.

POPULATION GENETICS/GENOMICS

The study of fungal population genetics, which explores the diversity and history of alleles (genes or genetic loci) in a species, has proven valuable in a number of ways. With respect to pathogenesis, for example, population genetics can reveal where an organism arose, how it evolved during periods of high and low disease rates, and the history of the organism and its transmission and distribution habits.

In fungi, as in all other classes of organisms, evolution works at the population level, and natural variations within populations play a major role in the evolution of new traits that can pose a threat or a benefit for humans. For therapeutic purposes, researchers must recognize variation in fungal populations in order to understand the pressures that drive the evolution of new species and traits.

Genetic variation within fungal populations is essential to their evolution, and knowledge of it is equally essential to understanding to how to control fungi that cause disease in animal or plants. In microbes, population genetic studies address population structure in species, modes of reproduction, gene flow among populations, migration to new geographic regions, population bottlenecks and expansions, and the forces of drift and selection in promoting adaptation. Until now, all population genetic studies have relied upon, at best, sequence data for a handful of genes. Now, with inexpensive methods of resequencing genomes, population genomics is upon us. Human population genomics is paying huge benefits in understanding human susceptibility to disease and is just beginning to touch fungal biology. Populations of genomes from yeast are now published and, for a few pathogenic fungi, small populations of genomes are now available (five for *Cryptococcus*, 15 for *Coccidioides*, including at least two for each of five populations). The tremendous amount of sequence data that population genomics will provide must also be curated centrally in fungal database to be of broad and general utility.

GENOMICS: PAST DISCOVERIES AND FUTURE POTENTIAL

Genomics—the study of all or part of an organism's genetic information—has had a profound impact on biology as a whole, introducing a new era of information-driven science. Many aspects of mycology, in particular, have been unlocked by sequencing the genomes of 100-150 fungal representatives of the estimated 1.5 million fungal species that exist. Although the sequenced representatives are heavily skewed toward human pathogens, these genomes are still unparalleled resources for revolutionizing the current knowledge of fungi, and they have thus far revealed new insights into evolution, sexual reproduction, gene families, gene acquisition, gene structure, and gene regulation. Genomics offers the chance to carry out science more rapidly and powerfully than ever before by pinpointing the gene "parts" of a fungus and opening a window on how those parts interact. Genome sequencing will

undoubtedly fuel improvements in bioremediation using fungi, industrial-scale fermentation processes, medical diagnosis, and disease therapies.

Because fungal genomes are relatively small compared to animal genomes, genome sequencing for these species would be cheap and easy, and technological advances are making genome sequencing less expensive and more facile every year. The tremendous diversity already discovered among the limited number of fungal genomes sequenced to date highlights the importance of seizing the opportunity of cheaper sequencing and conducting more comprehensive sampling of the rest of the fungal kingdom. Sequencing of fungal genomes has become "democratized" in a way, since the capability is now more accessible to labs with limited budgets. Where one genome sequence exists, additional individuals of the same species can be "resequenced" at 25X coverage by high-throughput methods for less than $10,000. Within five years, it will be possible to sequence a new fungal genome with similar coverage for only a few hundred dollars.

Data generation is no longer the primary limitation in genomics; the main challenge lies in the analysis and annotation of genomes and ultimately on the extraction of meaningful information. Falling prices for sequencing will make it possible to assess genome variation among multiple isolates and eventually turn genome sequencing into an assay. However, without a central database with a dedicated staff, these data will not be available to all researchers, and their potential will not be achieved.

Fungal Genomics: Future Potential

The application of genomics to study of the fungal kingdom will likely continue to bring about improvements in the management of fungal diseases, define new concepts in biology, open new opportunities in energy production, and reveal novel enzymes, secondary metabolites, and other biologically active compounds. Biotechnology, which often employs biologically active secondary metabolites from fungi, has benefited greatly from fungal genomics. Sequencing more genomes and experimenting with gene products using the knowledge gained from sequencing will inevitably reveal novel secondary metabolites that can be harnessed for use.

Eventually, researchers hope to be able to combine findings from expression, proteomics, metabolomics, and other analyses in order to measure the state of a cell in any set of circumstances. This capability is important, since manipulating or improving living things, including fungi, to suit human purposes requires a clear blueprint of the inner workings of a cell. In other words, we need to know how cells work before we can "fix" them.

Continued funding for fungal sequencing is critical considering the real possibility that genome sequencing will eventually lead to improvements in the detection, prevention, and treatment of fungal disease. Scrutinizing the interactions between the genomes of both the human host and the fungal pathogen may identify risk factors for fungal infections and ultimately enable development of tailored profiles

of a patient's susceptibilities to different fungi. Personalized medicine approaches like this avoid wasting resources on unnecessary treatments and enhance the quality of health care.

Genome sequencing of fungal pathogens also enables an in-depth understanding that can lead to tangible clinical benefits. Forming an information base about known pathogens will inevitably help the scientific community cope with the emergence of novel (but related) pathogens in coming years. The emergence of the human immunodeficiency virus (HIV) illustrates this possibility. When HIV was first identified, preexisting knowledge from other retroviruses helped the scientific community understand that virus and the ways in which it was able to evade and conquer the human immune system. Genomics can also reveal the regulation of gene expression (the production of proteins from the DNA instructions), which, in turn, aids an understanding of pathogenicity and how and when a pathogen's weapons are deployed against a host.

Comparative genomics, the detailed comparisons of the genomes of closely and distantly related species, is likely to contribute to understanding fungal gene organization and function. The identification of specific genes and their function in *Saccharomyces cerevisiae* has provided information that has enabled scientists to assign specific functions to human counterparts and provided an invaluable tool to identify genes and their functions in humans and animals. Also, the study of protein-protein interactions of yeast gene products offers a wealth of information regarding possible interactions that are also present in humans and animals. Recent studies of fungal genomes have uncovered the possibility that introns, small segments of DNA that do not encode proteins and were long thought to be "junk" DNA, could represent a novel type of mobile genetic element that may be lost or gained. The mechanisms by which introns are created would be of considerable general interest in biology. Recent comparative genomic studies have shown that introns are frequent and widespread in even the most basal fungi and that almost all intron change involves their loss. A reasonable hypothesis is that these elements originated as mobile, selfish elements that were co-opted by fungi to their present role as elements of genetic control via transcription. Comparative genomics can also be used to reveal the basis of virulence in fungal pathogens by identifying virulence factors (characteristics of the pathogen that allow it to infect a host) and pathogenicity islands (mobile genetic elements or gene clusters that play roles in pathogenicity). Comparative genomics also can be coupled with evolutionary biology to assess gene gain/loss, rates of evolution, and amounts of natural selection throughout the genome to identify genes that may be important to adaptation to pathogenicity.

Of course, fungi do more than cause disease. The approaches mentioned above can be applied with equal enthusiasm to fungi that have tremendous potential for use in the development of alternative energy sources, for remediation of chemical contamination, for the production of small molecules and therapeutics, and for biomass degradation. Fungi can also produce chemicals besides fuels (such as

enzymes, citric acid and itaconic acid), and biomass derived sugars could be used for all of these purposes.

All fungal genomes examined to date contain large numbers of genes for which the functions are completely unknown, but they may be useful in some way. In *S. cerevisiae*, a yeast that has been intensively studied for decades, as many as 1,000 genes remain uncharacterized with respect to their function. Considering the great value of the small number of characterized fungal genes, the uncharacterized fungal genes represent an intriguing untapped resource. Genomics will dramatically increase our ability to identify the species and genes encoding novel and improved enzyme activities, thereby expanding commercial applications and enhancing the sustainability of energy production.

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FUNGI TO TARGET FOR GENOME SEQUENCING

There are three areas where additional fungal genomic sequence is desperately needed: unrepresented phyla, close relatives of socially-important fungi, and population samples of these same fungi. Regarding unrepresented or underrepresented phyla, among the fungal genome sequences available now are representative species from four of the eight fungal phyla, including Ascomycota, Basidiomycota, Zygomycota, and Chytridiomycota, but most of the sequences originate from the ascomycetes, the most highly populated of the fungal phyla. The available zygomycete sequences originate from only three species (*Phycomyces blakesleeanus*, *Mucor circinelloides*, and *Rhizopus oryzae*) and the chytridiomycete sequences originate from only one species, *Batrachochytrium dendrobatidis*. The highest priority is for a second Chytridiomycota, the first Blastocladiomycota, the first Entomophthoromycotina, the first Zoopagomycotina, and the first Kickxellomycotina. Considering the significance of the many genomics-fueled discoveries outlined above and the paucity of fungal genome sequences available, sequencing more fungal genomes from a wider diversity of species is a high priority.

To better apply comparative, evolutionary genomics to pathogenic fungi, it is essential to have genome sequence from nonpathogenic species that are so closely related that nucleotide substitution has not yet saturated. For example, with *Coccidioides* species, the relative *Uncinocarpus* is too distant. What is needed are sequences from *Chrysosporium*.

Finally, to fully apply comparative, evolutionary genomics to socially-important fungi, population samples are needed. Here, 20 to 30 resequenced genomes per population would allow biologists to extract the most information about adaptive mutations that are most likely to be important to pathogenicity.

In all cases, whether the genomes are representatives of undersampled phyla, extremely close relatives of pathogens, or individuals in pathogen populations, the data must be made available thought a central, curated database.

The genomes that will benefit the most researchers should be prioritized for sequencing by assembling concerned scientists to work together to compile a proposal with defined rationales for the fungi of greatest interest. It is important to have representative sequences from each of the eight fungal phyla, but it would also be very useful to have selected groups of fungi that are particularly well sampled so that scientists may begin to understand the forces that drive evolution. Additional human fungal pathogens should also be sequenced, including, for example, *Sporothrix schenckii*, which is the only representative of the dimorphic fungal pathogens not currently in the process of being sequenced. This fungus is not only the causative agent of disease in humans, but is also related to plant pathogens of the Ceratocystis species.

Nonculturable fungi are another priority for genome sequencing. The ability to culture microorganisms is important, not only for diagnostic purposes in medicine but also to facilitate laboratory studies. Currently, a large proportion of microorganisms are nonculturable using traditional approaches, but in recent work researchers have successfully applied the knowledge gained from genome sequences to extrapolate culture conditions. For example, the genomes of two species of *Malassezia* associated with eczema and dandruff were recently sequenced, revealing that a gene that encodes a key enzyme for lipid biosynthesis is missing, a deficit that explains the strains' requirements for lipids when grown in the lab. Researchers have taken a similar approach with bacteria that previously could be grown only in the presence of their host; by sequencing these genomes they were able to extrapolate the appropriate conditions for cultivation. This strategy could be applied to nonculturable fungi like *Pneumocystis* species, which can currently only be grown in the lungs of infected animals.

Studies on basal, early diverging fungi and related organisms have much to teach us about the nature of the fungal family tree and about our own evolution from a unicellular ancestor. Genome sequences from key representative species can be used to determine whether microsporidia are fungi or the closest sister group of species to the fungal kingdom, thereby defining the earliest group of species that define the fungal kingdom. The microsporidia are a highly successful group of animal and plant pathogens with at least 1200 known species, of which at least 13 infect humans. They are obligate intracellular pathogens that cannot be grown freely in culture and have dramatically reduced genomes (as small as 2 to 3 MB, compared to 9 to 10 MB for the smallest free living fungi) that have undergone dramatically accelerated evolution. Further understanding of their relationship to fungi will be critical to understanding their pathogenic lifestyle and development of novel therapeutics.

The question of the origins and relationship of species to the fungal kingdom is of interest not only because it addresses fundamental questions about what is a fungus and what is not, but because it also has implications for the history of human evolution. Recent advances in molecular phylogeny have revealed that the animal and fungal kingdoms descended from a common ancestor (thought to have

been an aquatic, unicellular, motile organism with a posterior flagellum) roughly one billion years ago, indicating that animals and fungi share a common history and are more closely related to each other than previously thought. This area is currently the focus of a genome initiative addressing the origins of the evolution of multicellularity, with a focus on the genomes of 10 organisms, including three basal fungi (*Allomyces macrogynus*, *Spizellomyces punctatus*, and *Mortierella verticillata*), a species in a sister clade to the fungi (*Nuclearia simplex*, a member of the Nucleariidae, a sister group to the fungi), and representatives of the sister clade to the metazoa (including species in the Choanoflagellates (such as *Monosiga brevicollis*) and also the *Ichthyosporea* and *Capsaspora*).

PROPOSAL TO ESTABLISH A FUNGAL GENOME DATABASE

There are currently 70 complete fungal genome sequences distributed among numerous public and private databases, and it is likely that genome sequences will increase exponentially over the coming months and years. These sequences represent a wealth of information and a massive investment of money and time, but for a number of reasons they have only been explored superficially. The most significant obstacle to progress in fungal genomics is the lack of a centralized database in which genome sequences can be assembled, catalogued, and compared. Researchers involved with fungi must focus efforts on developing a comprehensive fungal genomics database in order to make the vast quantities of sequence data more available and to enable the field to fully capitalize on the promise of genomics. A single "Fungal Genomes Database" would offer one-stop shopping for the kingdom-wide data the scientific community needs and would make this information available to the broadest possible audience.

The Saccharomyces Genome Database (SGD)/Candida Genome Database (CGD) is extremely well-established, well-supported, and well-maintained and serves as a key resource for the scientific community concerned with yeast biology. A Fungal Genomes Database should be modeled on the SGD, while adding expanded apparatuses for comparing sequences and a user-friendly, nested design so that users may access only the level of information they desire.

The Fungal Genomes Database should include:

- Fungal genome sequences, including sequences acquired by "low pass" sequencing,
- Annotation and curated gene information,
- Links to the relevant literature,
- Structural data,
- Transcriptome data,
- Proteome data,
- Mutation and gene deletion data,
- Metabolic pathways,

- Metabolomic data,
- Comparative genome hybridization data,
- Mechanisms for comparing the biology of different strains in the database,
- Clinical information (including patient population information and minimum growth-inhibitory concentrations for effective antifungals),
- Plant pathogen information (including the host plant species and cultivar),
- Isolation data, and
- A wiki-style interface to allow users to add and annotate data.

Genome sequence data from sources outside the U.S. are sometimes overlooked. International data should be made available in the proposed Fungal Genomes Database.

Organizations with the expertise necessary to create and maintain a database like the one proposed here include the SGD/CGD, the Broad Institute, the Sanger Institute, the Munich Information Center for Protein Sequences (MIPS), and the J. Craig Venter Institute (JCVI), formerly the Institute for Genomic Research (TIGR).

FUNGI AS POTENTIAL BIOWEAPONS

Recent years have seen a rise in the incidence of international terrorism, and heightened concerns about national security have fed fears of bioterrorism. Fungi are a particular concern as potential bioweapons, since they possess properties that make them easy to generate and deploy and certain strains pose threats to crops and humans alike. Unlike such well-known bioweapons as *Bacillus anthracis*, fungal spores do not need to be "weaponized" for aerosol dissemination since natural selection has already done so. Terrorists could conceivably use fungi to take human lives or destroy crops or livestock. An attack on crops would inevitably impact the food supply and imperil the lives of humans and animals, and specific contamination of soil could render it unsuitable for cultivation for generations. Fungi can be engineered to express immune modulating cytokines that would enhance their virulence for human hosts. The extent of the threat from fungal bioweapons is global, and the potential economic impact of a well-designed and well-executed attack is enormous.

Fungi present a number of unique features that make them particularly manageable and threatening in the hands of a determined antagonist, including:

- Pathogenic fungi are inexpensive to acquire; often they can be isolated directly from soil or contaminated humans, plants, or animals.

- Many fungi are easy to culture in large quantities, and they do not require the use of technical equipment. Large quantities of spores of *Histoplasma* species and *Coccidoides immitis*, for example, would be easy to cultivate.

- Fungal spores are easy to disseminate over large territories without the need for weaponization or biological warfare engineering.

- Fungal spores resist desiccation, irradiation, and heat, and they have the potential to be disseminated by aerosol, so, in a sense, they are naturally weaponized and ready to deploy. As opposed to bacteria, many fungi would require few or no modifications before use as a weapon.

- Many fungal diseases are not easily or immediately detectable. This long incubation period is an advantage in a bioweapon, since national security and public health authorities would be slower to react to an outbreak that does not, at first, appear to be the result of a deliberate attack. Also, victims of a slow-developing fungal disease could delay treatment and other individuals would be infected from the contaminated environment in the interim.

- A number of fungi pose transkingdom threats and may be used to sicken both humans and important food crops. Such fungi would also be more easily spread among the target populations, since multiple hosts would shed and circulate more pathogens than a single host. *Fusarium* species, *Alternaria* species and *Cladosporium* species can cause disease in both plants and humans. Perhaps other fungi could be added to this list with the aid of genetic engineering.

The bioterror threat from fungi is not limited to their role as pathogens; fungi may also be used to manufacture fungal toxins for use against the public. Aflatoxins, for example, are potent fungal toxins that not only affect human health, they also exhibit toxicity against crops. In humans, aflatoxin is absorbed through the lungs, where it induces acute and chronic toxicity, and its effects have been observed in the context of a few poisoning incidents in the past. Widespread aflatoxin poisoning occurred in western India in 1974 and in Kenya in 1981 and 2004 when improperly stored corn became infested with aflatoxin-producing strains of fungi, and in 2005 aflatoxin-tainted dog food killed numerous animals in the eastern portion of the U.S. There are some reports that the Iraqi government was working on aflatoxin in its bioweapons program. Other toxins that pose a threat as weapons include the mushroom toxins amanitin and ergotoxin. Hallucinatory fungal compounds like those found in "magic mushrooms" could, conceivably, be used as weapons against the general public or the military if they were introduced into the food chain or water supply. On a local scale, the effects of certain fungi on allergic responses, including *Stachybotrys*, could also be exploited.

In the U.S., the Department of Health and Human Services (H.H.S.) and the Department of Agriculture (U.S.D.A.) have each promulgated lists of pathogens they think pose risks as potential bioweapons. These "select agents" are grouped according to the threat each represents; categories A and B organisms pose the highest risk and category B organisms pose less risk. The rules and restrictions for laboratories handling these category A and B organisms are onerous, so work with fungi on these lists involves significant investments of time and resources and often hiring or firing of lab personnel. For example, expensive containment facilities are required to work with category A and B organisms, only U.S. citizens and green card holders are approved to handle the organisms, and anytime a scientist leaves employment at the lab the locks must be changed. Moreover, certain organisms, including *Coccidioides*, are classified as select agents, but, until recently, have not benefited from federal funding for research on biological warfare agents. This forces researchers who want to study *Coccidioides* to secure the additional funding required to comply with the regulations, but the funding opportunities for *Coccidioides* are no greater than for any other pathogen.

A number of fungi are included on the lists of potentially dangerous organisms set forth by H.H.S. and U.S.D.A. However, some individuals and organizations involved in national security have not considered fungi to be viable threats. Close to 1.7 billion dollars have been designated for the study of organisms of biological weapon potential, but none of this money is designated for the study of fungal species. The potential for fungi and fungal toxins to be used as weapons is woefully underestimated and understudied, and the field needs advocacy to make fungi eligible for bioterrorism funding. Recently, *Coccidioides immitis* and *Coccidioides posadasii* have been added to the NIAID Category C Priority Pathogen list, illustrating at least some appreciation of the potential danger associated with these pathogenic fungal species (http://www3.niaid.nih.gov/topics/BiodefenseRelated/Biodefense/research/CatA.htm).

EDUCATION, OUTREACH, AND COLLABORATION

Despite the integral role fungi play in human health and disease, their devastating effects on agriculture, and their promise for use in industry, mycology still struggles for recognition as a vital and necessary field of study. The impact and importance of fungi is occasionally even neglected by microbiologists. Fungal biologists are poorly represented in many national advisory bodies or honorific groups, such as the National Academies of Sciences, and mycology is often an afterthought in microbiology and immunology courses.

Fungal diseases receive less attention from the medical community than most bacterial and viral diseases, in part because of a lack of statistics about their incidence. Unlike many other important diseases in the U.S., fungal diseases are not reportable, and in developing countries, where diagnostic tools are either poor or completely unavailable, there are almost no data about the incidence of fungal disease. The general public is largely unaware of the variety and severity of fungal infections that may afflict them, possibly because there are few well-publicized and alarming outbreaks of fungal infections (unlike bacterial infections, such as multi-drug resistant *Staphylococcus aureus* or viral infections like HIV).

The ability to effectively diagnose and treat fungal disease has suffered because fungal diseases are off the radar for the public, regulators, and the medical community. Charitable foundations find little reason to fund efforts to stamp out fungal diseases because the data substantiating the importance of these diseases do not exist. Similarly, a lack of fast and accurate diagnostics for fungal infections leads many doctors to treat their patients empirically, without diagnosis, employing large doses of potent antifungals until infections resolve. As a result, many hospital pharmacies spend one third of their budgets on antifungal drugs—a fraction well out of proportion to the number of patients suffering from fungal diseases.

The public should be more aware of the impact of fungi on their lives. Government officials and funding agencies seem to have little awareness of the fungal kingdom and its influence on public health.

Scientists involved with fungi need to establish collaborations with information translators to communicate about this important group of organisms. Journalists, for example, must be made aware of the basic differences between fungi, viruses, and bacteria. These are important and engaging distinctions to convey to the public so that they may better understand the prescribed treatments for their infections. A fungal infection needs treatment for an extended period, while many common viruses need or have no therapy, and a bacterial infection needs relatively short treatments.

Other interesting examples that will help mycology engage the public in developing an appreciation of the impact of fungi include:

- The chytrid attack on frogs has had a profound effect on amphibians. This is a compelling example in which amphibian species have actually gone extinct. Amphibians may be facing the greatest loss of diversity since the extinction of their equally charismatic cousins, the dinosaurs. In addition, there may be links to climate change. The frog pathogen is unlikely to become infective to humans because chytrid fungi need free water in which to disperse their flagellated spores, which lack a cell wall and thus die when desiccated. Also the particular chytrid that infects amphibians, *Batrachochytrium dendrobatidis*, is temperature sensitive and ceases growth at 28 C, almost 10 degrees Centigrade below human body temperature. However, other chytrids are associated with the stomachs of ruminant animals, and there is a recent report of chytrid DNA sequences found in the mouse gastrointestinal tract, and thus it is conceivable that chytrid fungi may already be associated with humans and be unrecognized commensals or pathogens, or might emerge to infect humans or other animals.

- The importance of fungal infections in cancer patients. Most people do not know that patients with cancer may die of infection (including, prominently, fungal infections), and not of the cancer itself.

- The high frequency of fungal infections in very low birth weight babies is troublesome.

- Common fungal infections, such as athlete's foot, impact the general public.

Ignorance and erroneous information about fungi abounds. The mycology community needs to agree upon the terms to use to inform the public. It may be advisable to assemble a focus group to identify the most effective way to explain and educate the public about fungi.

TRAINING NEEDS

There are a few identifiable weaknesses in mycology training today. Improving connections between mycologists and bioinformatic specialists and boosting training in classical mycology, a field that is currently lagging behind other areas of expertise, are of particular concern.

Mycology is overwhelmed with data, and the field is in search of ways to use it effectively. There is a great need to connect mycologists with bioinformatic specialists to improve education and training in mycology. Students and scientists alike need training in how to use databases and exploit the available resources in fungal genomics. With these tools in hand, mycologists will be prepared to

capitalize on the promise of genomics and begin to uncover the function of the vast number of genes that have yet to be characterized. The summer course in Molecular Mycology at the Marine Biological Laboratory in Woods Hole, Massachusetts, offers one opportunity to train scientists in mycology and pathogenesis that could extend to fungal bioinformatics.

There is a clear need to train more fungal physiologists and classical mycologists to maintain a pool of expertise in these fields. With respect to education and training, fungal physiology and classical mycology are losing ground to other, more recent branches of molecular mycology. Mycology must retain its knowledge base in classical mycology, lest scientists be forced to rely solely on molecular typing to identify environmental and clinical fungi, a technique that can give results that are inconsistent with biology. The field also needs to train experts in systematics who know fungi and can maintain culture collections.

Lastly, mycology is badly in need of support for training. In many of the funding agencies, there are sizeable gaps between the funding afforded to fungal biology and that afforded to other, comparable branches of biology.

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